ORCAS meeting on Nanosensors
October 16, 2006

On October 16, 2006, the Oak Ridge Center for Advanced Studies hosted a one-day meeting bringing together scientists from the Environmental Protection Agency (EPA) and National Institute for Environmental Health Sciences (NIEHS) and nanotechnologists to discuss their shared interest in developing novel nanoscaled analytical instrumentation for specific applications. EPA and NIEHS investigators were asked to articulate the challenges they encounter as it relates to identifying, characterizing, and monitoring regulated chemical species in vivo and in situ. In response, the invited nanotechnologists were allowed to make the case for a wide spectrum of promising nanoscaled analytical tools. The meeting served as a follow-up to a larger one held in April 2006 at the EPA campus in Research Triangle Park (see http://orcas.orau.org/epa/default.htm).

The Case for Nanotechnology – *Commentary by Michael Strano (Asst. Professor, University of Illinois- Urbana)*

The case for nanotechnology in most sensing applications varies both with the type of material used in its design and the physical and chemical properties of that material. For example, the dimensionality of a given material and recent advances in fabricating reduced dimensional materials has been a major influence on nanosensor design. One-dimensional materials that restrict electrons to a single dimension and to a single path of motion have been used in the fabrication of nanowires, nanotubules and nanopolymer based sensors. As sensors based on electrical resistance changes, these represent the absolute limit.

Fluorescence-based techniques are some of the most powerful molecular detection methods available. Single molecule fluorescence analysis is a now routine assay in scientific laboratories. For optical fluorescence-based sensors, there are classes of nanoparticles that exhibit extremely enhanced photostability in fluorescent emission. Single-walled carbon nanotubes are infinitely photostable at moderate light fluxes, for example. This means that for the first time, new types of sensors can be devised with extremely long operational lifetimes. This is not possible with conventional fluorophores. Some nanosystems emit light at longer wavelengths where few conventional materials operate whereas few conventional materials do so.

The human body is particularly transparent to near-infrared light in a narrow region of the electromagnetic spectrum. These systems will form the basis of novel detection technologies that can operate in strongly scattering media where fluorescent spectroscopy is limited.

Nanoparticles can also possess features that are commensurate with biomolecules and other important macromolecular analytes. Electrodes that are narrow enough to fit or conform to biological structures should be capable of transducing subtle changes in these structures, as several pioneering efforts are already demonstrating.

It has been pointed out that generally the detection limit of a sensor scale approximates the cube of its characteristic length. So smaller sensor elements mean lower detection limits generally. However, other problems become important, including diffusion to the sensor element, as length scales are reduced.

Typical Nanosensor Characteristics

The potential of highly selective nanosensors as a widely utilized and integrated analytical tool could have major implications in our environmental detection schemes. The ability of a putative nanosensor to discriminate between the desired analyte and a host of potential contaminants is a desirable characteristic for many studying human exposure events. The sensitivity is defined by the smallest concentration of an analyte which a nanosensor can detect; this is represented by the lowest measurable response whose limits do not include zero. The term 'sensitivity' as it relates to nanosensors may indicate the degree of discrimination between measurements at any concentration level.

Sensor shelf-life

The shelf-life of the nanosensor is dependent on the lifetime of the sensing layer and not so much on the sensing platform. The nanosensor lifetime will vary as a function of the sensing layer. For example, bioreceptors (antibodies, enzymes, lipid layers) are limiting factors because of their inherent short life span under non physiological conditions. On the other hand, aptamer- and polymer-based sensing layers have been used in an effort to extend the lifetime of the device.

Real-time detection

Real-time detection is a common feature of nanosensing technology. The nanosensors described in the meeting all operated on a time scale ranging from seconds to minutes.

Binding

Nanosensing platforms built on analyte-receptor binding activity can be an effective mode for detection in air or fluid mediums. The binding mechanisms can be described as reversible—requiring little or no surface treatment to return the sensor to its steady state—or irreversible where analyte binds with high affinity such that surface treatment is required to remove the bound substrate. This property may be more effective in an environment where high discrimination is needed in the face of a variety of extraneous compounds.

A. **Detecting Cyanotoxins** ***(****Presented by Karl Jensen, EPA****)***

Problem: Cyanobacteria and their toxins are candidates for regulation under the Safe Drinking Water Act, which requires assessment of both their occurrence in the national water supply and their toxicity. Assessing occurrence is complicated by the complexity of the mixtures of toxins produced by blooms. Assessing toxicity is complicated by the varied potency of the diverse toxins comprising these mixtures. To address these challenges, an Interagency Workgroup recommended (1) the development of "broad spectrum" detection methods sensitive to multiple analogues that comprise major classes of cyanotoxins, and (2) the application of such detection methods to standardize cyanobacteria bloom extracts for use in health effects studies. Nanosensors sensitive to broad classes of cyanotoxins have the potential to provide an approach addressing these challenges as well as providing a linkage essential to an integrated approach to risk assessment.

Discussion: The AquaSentinel system was described by Elias Greenbaum of ORNL. This system is based on the natural fluorescence of freshwater algae and modifications in the characteristic fluorescent profile produced by toxins. Based on a number of examples of acute exposures, it was proposed that a database could be developed of toxin-specific signatures. Toxin identification and quantification could potentially be achieved by using an extensive database of signatures to 'deconvolute' complex real-world data.

B. **Assessing Nervous System Integrity** *(Presented by Karl Jensen, EPA)*

Problem: Assessing the integrity of the nervous system is an important component of toxicity testing. Neurotoxicity assessment currently relies on complex, skill-intensive, time consuming and expensive behavioral and neuropathologic assessments. The integration of nanosensors with in vivo imaging has the potential to dramatically reduce the time, cost, and number of animals necessary to obtain essential information on the neurotoxic potential of agents subject to toxicity testing. One potential approach is the application of nanosensors that recognize GFAP (glial fibrillary acidic protein), a widely accepted histopathologic and neurochemical marker of nervous system injury. Other approaches may focus on detecting reactive microglia, which would include detection of an inflammatory response. The use of nanosensors for in vivo detection of alterations brain development would be particularly valuable. In vivo developmental neuroimaging that relies on visualization of intrinsic markers such as cytoskeletal or synaptic proteins (or lipids such as cerbrosides) is a likely application for designing nanosensors with unique vectoring properties that selectively target the brain.

Discussion: Nanosensors have been developed that serve as enhanced contract agents for MRI at the cellular level that are based on super-paramagnetic nanoparticles with phospholipid micelle coating. While coupling such nanosensors with antibodies specific for nervous system proteins might be feasible, a significant limitation for their application to in vivo whole animal imaging is the relatively small amount of nanosensors likely to reach the brain. The few studies of tissue distribution to date indicate the majority of

metal nanoparticles tend to be rapidly excreted. The bulk of what remains tends to be found in the liver and kidney. What does remain, appears to persist.

Several possibilities were discussed that might overcome the limitation of the amount of nanosensing particles reaching the brain.

(i) The use of "diamond like" carbon nanoparticles (idea presented by Jim Davidson of Vanderbilt) which does not appear to bind to components of blood or induce an inflammatory response, and thus, might have a more likely possibility of reaching the brain.

(ii) The use of nanosensors based on PBR binding (idea presented by Gang Bao of Georgia Tech) might be of value to increase the resolution of persistence of signals with PET imaging.

(iii) Another approach discussed was the use of transgenic animals in which ferritin (and possibly transferrin) genes were linked to promoters for GFAP. The coupled expression of these two genes has been demonstrated to result in sufficient selective iron accumulation for visualization by MRI within cells in vitro. The coupled expression of the genes with reactive astrocytes may result in sufficient iron accumulation to aid in the visualization of 'hot spots' of GFAP expression associated the astrocytic response to injury (analogous to the detection of iron rich amyloid plaques that were found in mice models of Alzheimer's disease reported by several labs.)

(iv) Another approach that was suggested for detecting brain injury in live rodents with in vivo imaging was to assess alterations in brain hemodynamics. While the approach did not involve MRI, it provides information analogous of the method "functional MRI" used to map region specific brain activity.

C. **Development of Nanosensors to Link Exposure to Toxicity via Mode\Mechanism of Action** *(Presented by Kevin L. Dreher, US EPA, ORD, NHEERL)*

Problem: The "idea" for this proposal has multiple origins. First, oxidative stress is a common mode of action for a variety of environmental pollutants\stressors and is a significant causal mechanism of their underlying pathology. Secondly, a significant body of evidence in particulate air pollution health effects research has indicated that particle "reactivity" or ability to generate oxidative stress correlates with its toxicity or adverse health effects. Thirdly, information is accumulating that surface properties including surface reactivity regulates the toxicity of a number of classes of manufactured nanomaterials. Finally, PM hazard identification research has demonstrated that particle size and chemical composition regulate PM health effects and thus may represent a better exposure metric when compared to mass.

Discussion: Nanotechnology offers for the first time a unique opportunity to link exposure to effects. Research into the oxidative chemistry or “reactivity” of air

particulate pollution has identified at least two, possibly more, pathways associated with oxidative stress. Developing nanosensors to detect, monitor, and measure these pathways in ambient air in conjunction with employing the same nanosensors in toxicological studies will allow one to directly link exposure to PM "reactivity" to health effects. The development of "reactivity" nanosensors could mirror nanomedicine technology where drugs that sense their environment and respond accordingly to release drugs or detect alterations in metabolites have been produced. Such nanosensors could easily be incorporated into multiplex air monitoring platforms that employ nanosensors to measure other NAAQS regulated pollutants. This research would develop in several stages:

Stage I Complete understanding of ambient air PM and emission source particle acellular oxidative chemistry and cellular effects. It should be noted that a significant amount of research has already been done in stage (I).

Stage II Development of reactivity/oxidative stress nanosensors consistent with data from stage (I).

Stage III Beta testing an ambient monitoring and toxicity testing case to confirm proof of principle.

Stage IV Full test scenario with exposure monitoring using reactivity nanosensors and associated toxicity test employing them. Additional application of these reactivity nanosensors can also be extended to probing the surface reactivity of nanomaterials in order to relate this property to their toxicity.

Minimal Disciplines Required: Redox Chemist, Nanomaterials Chemist, Biologist/Toxicologist, Aerosol Engineer in NAAQS Monitoring

D. **Exposure assessment for Large Scale Epidemiological Studies (e.g., National Children Study** *(Presented by Jim Quackenboss, EPA)*

Problem: Exposures to many environmental chemicals change over time and between locations where children spend time. For many chemicals personal monitors have used pumps to collect air samples on filters and sorbent materials, which are then analyzed to measure integrated average concentrations over some period of time (usually days or weeks). However, the size, weight, noise-level, and appearance of monitors maybe unacceptable to many study participants, especially children in child care or school settings. Valuable information on the locations and conditions (e.g., sources) where/when exposures occur and on the frequency and magnitude of transient (high-level) exposures is lost in longer-term integrated samples. Passive samplers (e.g., badges or sampling tubes) provide a way of collecting samples without pumps, but are limited in the types of analytes available at environmental concentrations, and may require longer sampling periods (e.g., 1-2 weeks) to obtain adequate samples in non-occupational environments. These require open faces or tubes for sampling, and have potential interferences from face velocity, temperature changes, and humidity levels. Small

devices (e.g., about the size and appearance of cell phones) that could provide some temporal information (e.g., 5 min – 1 hr averages) and be downloaded remotely could provide an attractive alternative for personal and micro-environmental measurements. The ability to include multiple agents from a given medium would allow for fewer measurements to be taken, which would minimize the time for setting up and collecting samples in homes, and the number of samplers being worn or carried by a participant. Examples of the types of agents and media are given below.

The design of the National Children's Study NCS is based on hypotheses which relate exposures to environmental agents to major health outcomes of concern for children.[1] While environments are more broadly defined for the Study, potential sensor applications may include detection/quantification of chemical and biological agents, including the following:

- Air pollutants (e.g., particulate matter, nitrogen dioxide, ozone and carbon monoxide);
- Volatile organic compounds [VOCs] in air (e.g., formaldehyde, benzene, vinyl chloride, other aldehydes, acrolein, and ketones) and drinking water (e.g., trihalomethanes);
- Semi-volatile and non-volatile organic compounds (e.g., include organophosphate [OPs], carbamate, and pyrethriod pesticides, herbicides, polycyclic aromatic hydrocarbons, phthalates, halogenated phenols, alkyl phenols, and environmental tobacco smoke) in air, house-dust, soil, drinking water, food, urine (metabolites) and blood;
- Allergens (e.g., cat, dog, mouse, rat, cockroach and dust mite antigens), molds, and pollen in dust samples;
- Bioaccumulative (e.g., lead, mercury, and cadmium) and non-bioaccumulative (e.g., arsenic, chromium, manganese, nitrate, and perchlorate) inorganic chemicals in air, dust, drinking water, food, urine (non-bioaccumulative) or blood (bioaccumulative).

Multi-analyte analytical instruments requiring small sample volumes.
There are a number of water samples that are proposed for homes, depending on whether they are private wells in rural areas or are on municipal water systems. These include samples collected for disinfection by-products (VOCs and haloacetic acids), metals, coliforms, nitrate, perchlorate, and various classes of pesticides. Each of these samples takes time to collect and then must be stored and shipped back to a laboratory for analysis. Many of the analyses must be done within specified time periods to provide valid results. If multi-array sensors could be used with a single water sample shortly after collection, then the numbers of samples and independent analyses could be reduced.

There are a number of chemicals of interest in blood samples, but the quantity of blood available is limited (especially from young children). For the NCS, this includes analyses for:

- Specific IgE's for cat, dog, cockroach, dust mite, fungi, and mouse/rat urine;
- Glucose and HgbA1C (hemoglobin aduct for longer-term glucose);
- Exogenous chemicals such as PCBs, pesticides, PBDE, Perfluorinated compounds (PFOA, PFOS), dioxins/furans, lead, and mercury. Analyses of blood samples for non-persistent pesticides (OPs, carbamates, and pyrethroids) are difficult given that these are

[1] See http://www.nationalchildrensstudy.gov/research/hypotheses/ for specific hypotheses and major exposure and outcome domains

rapidly metabolized and are usually present at low concentrations in the blood. However, they are more specific for exposure to that compound, while the metabolites measured in urine may reflect exposures to both that compound and metabolites in environmental media (e.g., food).

Temporal variations in urinary metabolites could be assessed using sensors and the results could be stored for later retrieval or remotely transmitted to indicate that an acute exposure event has occurred, which could trigger collection of environmental samples to help identify the sources or pathways leading to these exposures. For the NCS, urine samples will be analyzed for several exogenous chemicals, including alkyl phenols (Bisphenol A, nonylphenol), mercury (inorganic), arsenic (speciated), perchlorate, halogenated phenols (PCP), phthalates, atrazine, and non-persistent pesticide metabolites.

Screening environmental and biological media for higher concentrations.
Conventional analyses of the chemical agents and media identified above usually try to obtain levels of sensitivity as low as possible in order to provide information on the full distribution of concentrations in environments or for populations, or to demonstrate compliance with associated regulatory limits. However, the cost for these analyses has a large impact in a large longitudinal study, and may limit the numbers and types of samples that can be collected and the numbers of time periods that can be observed for each study participant. For an epidemiological study like the NCS, it would be desirable to detect levels that are of concern for potential acute health risks or to identify more highly exposed individuals and environments (e.g., upper 75^{th} or 90^{th} percentiles of the distribution). This information could be used to examine differences in the rates of adverse outcomes occurring among more "exposed" populations, in comparisons with others in the cohort. Concerns about potential measurement error and misclassification could be addressed by focusing analyses by conventional methods on those with "high" concentrations and samples of others. Rapid screening methods for multiple analytes, or classes of analytes based on similar mechanisms of action (e.g., cholinesterase inhibition), could provide opportunities to identify/classify those with higher exposures and trigger additional environmental (or personal) sample collections for analysis using conventional methods. There are some challenges with making these measurements in the field, including matrix effects and using simpler sample preparation methods. However, the use of such methods could help identify sources of these exposures and possible ways to reduce exposures, as well as identify samples that are more likely to have measurable concentrations by conventional methods.

Discussion: Suggestions from scientists at the ORCAS Nanosensor Meeting.
During the initial presentation by Thomas Thundat, the possibility of using multiple coatings for different cantilevers was suggested as a way to develop multi-analyte sensors and a hand-held devise for explosives detection was shown. This includes a small pump and could probably be reduced in size, while still including some memory and wireless communication capabilities. His presentation also mentioned antibody-antigen coatings that were very selective. This may hold promise for some chemicals, especially where immunoassay techniques have been developed, or for analysis of specific antigens in air or from house dust samples. In addition, the NCS is going to be using blood samples to

identify IgE for specific antigens (as well as chemicals), and reductions in the blood volumes required would be helpful.

The discussion on identification of microbes (Potential Application G; Richard Zepp) was for surface waters, but may also have applications to identification of coliforms in drinking water. The conventional tests for this require analysis within 24-hr, which poses a challenge for study operations that are spread out to various locations and add to shipping costs. While not discussed, the potential of using similar techniques to measure endotoxins in personal air or house dust would also have applications, especially given recent evidence showing differences between personal measurements and room air concentrations[2].

The development of personal exposure monitors for criteria air pollutants (Potential Application I Stephen McDow) included discussion of multiple sensors and channels. Tuan Vo-Dinh commented on previous work involving passive samplers for gaseous air pollutants, and EPA's personal monitoring for carbon monoxide using electrochemical sensor-based monitors. There may be opportunities to improve the performance of these technologies and miniaturize multi-sensor units that could be easily carried by participants and then download data remotely. There was also some discussion about the reversibility of sensors as being a general concern, and how detection limits could be improved.

Opportunities provided by the NCS.

The National Children's Study is planned as a long-term effort to evaluate the determinants of children's health and wellbeing over various stages of growth and development. We need to consider how new technologies could be used to help improve the quality and representativeness measurements, while minimizing participant burden and costs. The Study will use a probability sampling design to represent children born in the US, including a wide range of geographic locations, environmental conditions, and demographic groups. There will also be a need to ensure that measurements are comparable over time, so that there will be an ongoing need to make inter-comparisons with more conventional sampling and analytical methods. This provides opportunities for moving nanosensors from the laboratory into real-world applications, and for field testing and then possibly deploying these to measure changes in environments over time in the context of a large-scale study that will be directly assessing the impact of exposures on health.

[2] Rabinovitch, N., Liu, A., Zhang, L., Rodes, C., Foarde, K., Dutton, S., Murphy, J., and E. Gelfand, "Importance of the Personal Endotoxin Cloud in School-Age Children with Asthma," JACI, 116:5(1053-1057), 2005.

E. **Apparatus for Identification of Microbes** *(Presented by Richard Zepp, EPA)*

Problem: Despite advances in water treatment, source water protection efforts, and the presence of several layers of local, state, and federal regulatory protection, many sources of raw and finished public drinking water in the United States periodically contain chemical, microbiological, and other types of contaminants at detectable and sometimes harmful levels (2001 NRC report "Classifying Drinking Water Contaminants for Regulatory Consideration"). The emergence of highly sensitive, highly selective nanosensors for real-time detection and identification of virulent organisms in recreational waters and drinking water supplies detection and removal, could have major implications in the development of existing and proposed environmental databases to assist in proactive identification and regulation. When implemented these devices can potentially be used to forecast spatiotemporal distribution and evaluate effectiveness of remediation (POTWs, BMPs, etc), preferably whole cell measurement.

Relevant Microbial Biometrics

- Typically sorbed to surface such as sediment or biofilm – not freely suspended in water.
- Typically cells > 200 nm in size.
- Lifetimes: few minutes to weeks.
- Detection needed: for beaches E. coli 240 cfu/100 ml; enterococci 61 cfu/100 ml; real time especially during day.

Discussion: Richard Zepp suggested the development of nanoscale sensors for measuring microorganisms that would sense virulent species and provide other information about microbial functioning in ecosystems (e.g., in N cycling such as denitrification). The sensors could be incorporated on biochips that permit many tests to be performed at the same time in order to achieve higher throughput and speed. Whole cell measurements would be preferable. The nanosensors would be used to detect virulent organisms in recreational waters and drinking water supplies at concentrations required for public warnings (e.g., 61 cfu/100 ml for enterococci at beaches). Other nanoscale sensors would identify microorganisms involved in biogeochemical cycling in ecosystems, including nitrogen and carbon cycling. For example, these sensors would track expression of key prokaryotic genes and biochemical pathways that are important in mediating nitrogen cycling in ecosystems. The sensors would be used to determine the spatiotemporal distributions of the microorganisms and to evaluate the effectiveness of ecological remediation activities.

F. **OZONE AND PM SENSORS** *(Presented by Steve McDow, Human Exposure and Atmospheric Sciences Division, EPA)*

Problem: Ozone and particulate matter are the regulated air pollutants most frequently in non-attainment of national ambient air quality standards. A significant uncertainty in understanding health risks from ozone and particulate matter is quantifying personal exposure and its relationship to that from ambient-based measurements. Health effects have been noted for both long-term as well as short-term exposures for these pollutants. Integrated measurements for these pollutants are readily performed by stationary-rack mounted and/or Federal Reference Methods having a high degree of sensitivity. To date, there has been little progress in development of highly sensitive, portable, mass-based sensors for these pollutants. However, accurate personal monitoring for these pollutants is needed because some studies have shown that there can be poor correlation between ambient-based measurements and those obtained from residential or personal monitoring. Thus, sensitive, lightweight, portable sensors that can measure these pollutants real-time (maximum 10 minutes) in a person's breathing zone and that are coupled with physical activity sensors to allow accurate assessment of energy expenditure are needed.

Commercially available monitors are limited by time resolution, monitor size, or both. Ongoing exposure studies in the Human Exposure and Atmospheric Sciences Division at EPA depend on a nitrite-coated Ogawa Badge to monitor ozone exposure. Average ozone concentration is estimated by measuring the extent of the nitrite-ozone reaction. It is a suitable size for wearing, but is limited to 24 hour measurements to allow sufficient reaction for reliable measurement to occur. Ozone can be measured in real time by either chemiluminescent reaction with nitric oxide or UV absorption, but commercial instrumentation is too bulky for use as a personal monitor.

Current exposure studies for particulate matter also rely on personal monitors limited to 24-hour measurements. Like ozone, measurement needs for particulate matter include a convenient size monitor and real-time measurement capability. In addition, particle size resolution and chemical speciation are also important because particle size and composition might strongly influence health impacts. Health effects are important but different for coarse (10-2.5 μm dia.), fine, and ultrafine particles. Personal monitors are currently available for measurement of total or size resolved (coarse and fine) particles, but are limited to 24-hour time resolution and not capable of chemical speciation. These are flow-through filtration devices for gravimetric measurements of deposited particulate mass. Commercially available real-time fine (< 2.5 μm dia.) and ultrafine (< 0.1 μm dia.) particle monitors operate by exposing particles to air supersaturated with water so that particles grow to a size that can be easily observed by optical scattering (condensation nuclei counter or condensation particle counter). Light-scattering technologies are also available and in use but these require standardization with filter-based methods and thus have an inherent limitation to their use.

The bulk constituents of atmospheric particulate matter are sulfate, nitrate, ammonium, organic matter, and elemental carbon. There is a strong need for sensors with real-time capability for measuring 1) these bulk species, 2) known hazardous or toxic components such as polycyclic aromatic hydrocarbons or heavy metals, 3) potential source markers

such as pyrolyzed sugars or petroleum markers like hopanes, 4) organic functional groups like carboxylic acids or aldehydes, or 5) physical or chemical properties that could be used as indicators of sources or toxic properties, such as fluorescence or absorbance.

Overall, the greatest needs for ozone and particulate matter sensors are short time-resolution (less than 10 minutes), convenient size (wearable), and for particulate matter, size resolution and chemical specificity.

Discussion: The ability to detect and monitor multiple analytes by less intrusive means is the foundation of most nanosensor designs. Thomas Thundat (ORNL) has developed a cantilever array sensing system the size of a dime, capable of detecting multiple agents based on its unique molecular signature.